

chickens on vitamine-restricted diet. The fourth generation after thirteen days' growth from February 12 to 25, 1918, showed the character of the original growth, especially the capacity to penetrate into the underlying tissues and to destroy them.

I will not omit to point out that the growth described here did not show bacterial contamination. The possible objection, that this growth which is very similar to those described by Rous,¹ 1910, *a* and *b*, and Rous and Lange,² 1913 (compare 1910, Fig. 5, 1910, Figs. 2 and 3, and 1913, Fig. 7) might be a "spontaneous growth," I will refute immediately by saying that the repetition of this series of experiments on a larger scale was successful. Only in two of twelve chickens, which had received (1) implantation of heart tissue, (2) tetheline injections into the implant, (3) vitamine-restricted diet, growths were produced, the other ten, in which one or two different factors are lacking, showed no protuberance formation.

The produced growth, the genesis of which I have outlined, seems to be a connective tissue growth. It is infiltrable, destructive and transplantable. Further cytological investigation, especially the solution of the question, whether the implanted embryonic tissue cells are really forming by their descendants part of the growth, will be necessary to name this transplantable growth correctly.

A detailed description of this growth and two others, together with their descendants, will appear later.

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Immunization of monkeys against poliomyelitis.

By H. L. ABRAMSON (*by invitation*).

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This work represents efforts made toward developing a practicable method of immunization against acute poliomyelitis. Highly potent monkey polio virus is attenuated by heat in two ways. In one case 5 c.c. of brain and cords in 10 per cent. saline

¹ Rous, *Jour. Exp. Medicine*, 1910, p. 285 and p. 397.

² Rous and Lange, *ibidem*, 1913, p. 651.

suspension from monkey poliomyelitis is subjected to 55° C. for one half hour and injected subcutaneously into monkeys on ten successive days. This represents the use of killed virus. In the other method the 10 per cent. emulsion is subjected to graded attenuation as follows: heated to 55° C. for one half hour on first two days; to 45° C. on third day; to 37° C. on fourth day and on the fifth day the material is injected unheated.

On the 20th day after the series of subcutaneous injections are completed, the treated animals are bled from the heart in order to test for the presence of neutralizing substances in the blood. On the following day the treated animals were tested by subjecting them to three to five intracerebral lethal doses of highly potent monkey virus. The sera of the treated animals were placed in contact with equal part of 5 per cent. emulsion of monkey virus at 37° C. for two hours and then in ice box over night. On the day of intracerebral test for treated animals, the serum virus mixtures were inoculated intracerebrally into normal monkeys. The same virus emulsion was used in the test inoculations and serum virus mixtures.

The results with killed virus injection method were not so good as with that of graded attenuation. In the former method only 3 animals were used. Of these two succumbed to test inoculations with the usual incubation and the third presented a delayed incubation of 12 days, but recovered with residual paralyse of both arms and legs. The serum of one of these completely neutralized virus; in another the incubation was prolonged to 18 days but animal recovered and now presents residual paralysis of legs; in the third the incubation was prolonged to 10 days and animal died. The incubation periods of two normal controls were 5 and 6 days respectively.

Of eleven animals subjected to injections by the graded attenuation method, none showed any ill effects from the treatment itself. Five out of eight subjected to the intracerebral test injection of from 3 to 6 lethal doses resisted successfully. Sera of all the animals tested demonstrated the presence of neutralizing substances. Three completely neutralized 5 per cent. emulsion of highly potent virus in proportion of 1 : 1. Four others delayed the four- and five-day incubation periods of normal controls to

from 10 to 25 days. To indicate potency of test virus two animals that had recovered from previous experimental infection and which presented residual paralyses succumbed as readily as the normal controls to the intracerebral injection of only three times the dose used to test the treated animals. Animals that have recovered from experimental infections have been found to be highly resistant to reinfection.

The latter method requires only a short time; the material is easily produced and gives a considerable degree of immunity. The test method is far more severe than that to which persons are subjected in the natural infection inasmuch as in this artificial test the defensive forces in the blood stream are circumvented, whereas in the natural infection the infective material would first come in contact with the neutralizing substances present in the blood stream before it could gain entrance to the central nervous system. The natural infection is far less severe than the experimental disease in which we may have a mortality of about 99 per cent.

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Persistence of immunity following toxin-antitoxin injections.

By **WILLIAM H. PARK.**

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During the past years, we have immunized a series of guinea pigs and a series of horses and a series of children with diphtheria toxin-antitoxin mixtures. The duration of the immunity presents certain points of great interest. So far as our observation goes, guinea pigs are never naturally immune; horses in the very great majority of cases are immune; adults and infants under 6 months are immune to 80 per cent., while very young children are non-immune to about 60 per cent. We find, in the guinea pig, that active immunity lasts for about nine months. In horses, we find the increased immunity, due to the injections, lasts from nine months to twelve months, at which time the horses return to their original amounts of natural antitoxic immunity.

In testing human beings from month to month, it was a matter of great interest to find whether the infants and children who be-